JSHC
Japan Society of Histochemistry and Cytochemistry
Established 1959

Acta Histochem. Cytochem. 43 (5): 115–121, 2010
doi:10.1267/ahc.10023

Immunohistochemical Examination for the Distribution of Podoplanin-Expressing Cells in Developing Mouse Molar Tooth Germs

Yuri Imaizumi[1], Ikuko Amano[1], Eichi Tsuruga[2], Hiroshi Kojima[1] and Yoshihiko Sawa[2]

[1]Department of Oral Growth & Development, Fukuoka Dental College and [2]Department of Morphological Biology, Fukuoka Dental College, 2–15–1 Tamura, Sawara-Ku, Fukuoka 814–0193, Japan

Received August 9, 2010; accepted September 9, 2010; published online October 15, 2010

We recently reported the expression of podoplanin in the apical bud of adult mouse incisal tooth. This study was aimed to investigate the distribution of podoplanin-expressing cells in mouse tooth germs at several developing stages. At the bud stage podoplanin was expressed in oral mucous epithelia and in a tooth bud. At the cap stage podoplanin was expressed on inner and outer enamel epithelia but not in mesenchymal cells expressing the neural crest stem cell marker nestin. At the early bell stage nestin and podoplanin were expressed in cervical loop and odontoblasts. At the root formation stage both nestin and podoplanin were weakly expressed in odontoblasts generating radicular dentin. Podoplanin expression was also found in the Hertwig epithelial sheath. These results suggest that epithelial cells of developing tooth germ acquire the ability to express nestin, and that tooth germ epithelial cells maintain the ability to express podoplanin in oral mucous epithelia. The expression of podoplanin in odontoblasts was induced as tooth germ development advanced, but was suppressed with the completion of the primary dentin, suggesting that podoplanin may be involved in the cell growth of odontoblasts. Nestin may function as an intermediate filament that binds podoplanin in odontoblasts.

Key words: podoplanin, tooth germ, odontoblasts, enamel epithelia, nestin

I. Introduction

Podoplanin is the antigen of the kidney glomerular epithelial cell, the podocyte. In a rat model of nephropathy by puromycin aminonucleoside nephrosis with severe proteinuria, the podocyte foot processes exhibit extensive flattening and podoplanin is selectively reduced to less than 30%. It is thought that podoplanin plays a role in maintaining glomerular permeability because morphological alterations of cell shape occur with selective loss of podoplanin in the nephrosis that accompanies proteinuria [3, 13, 18, 19]. Podoplanin is also a well-established lymphatic endothelial cell marker which is recognized by the D2-40 antibody, and is homologous to the T1α-2 gene which encodes the type I alveolar cell specific antigen. Podoplanin is first expressed at around E11.0 in Prox1-positive lymphatic progenitor cells and podoplanin deficient mice die at birth because of respiratory failure. Podoplanin deficient mice also have defects in lymphatic formation with diminished lymphatic transport and congenital lymphedema [3, 4, 7, 10, 14, 21, 23–25, 27, 28, 30, 31]. Furthermore, there have been reports on podoplanin expression in mesothelial cells [23, 24], epidermal basal layer cells [14], choroid plexus epithelial cells [37], thymus type I epithelial cells [3], and immature cells like fetal germ cells and developing Sertoli cells [11, 32, 40]. Since podoplanin is a mucin-type transmembrane protein it is thought to be involved in the development and homeostatic maintenance of several kinds of cells by its extensive O-glycosylation with a high content of sialic acid [11, 30–34].

In oral tissue we have recently reported the expression of podoplanin in mouse salivary gland myoepithelial cells [8, 9, 30]. The immunohistochemical study revealed that myoepithelial cell processes on the terminal portion of

Correspondence to: Yoshihiko Sawa, Department of Morphological Biology, Fukuoka Dental College, 2–15–1 Tamura, Sawara-Ku, Fukuoka 814–0193, Japan. E-mail: ysawa@college.fdc.net.ac.jp

salivary glands express podoplanin. In an immunoelectron microscopic study podoplanin localization was found at the Golgi apparatus binding to endoplasmic reticulum near nuclei in the cytoplasm of myoepithelial cells and at the myoepithelial cell membrane, indicating that podoplanin is a marker protein of salivary gland myoepithelial cells. On the other hand, we have also reported the expression of podoplanin in apical bud cells at cell-cell contacts in the adult mouse incisal tooth [29]. Podoplanin expression in mesenchymal cells has been reported in cells exhibiting morphological diversity: osteocytes and osteoblasts [36], prostate myofibroblasts [26], and follicular dendritic cells [16, 38]. Therefore, it was thought that the expression of podoplanin is also induced in developing tooth germ cells with differentiation and morphological changes.

In this study, nestin was used to identify neural crest-derived mesenchymal cells. Nestin is an intermediate filament that is useful as a neural crest stem cell marker [1, 12, 22, 29, 35]. Nestin is expressed in the central nervous system stem cells from the neural tube and in immature skeletal muscle cells, and is replaced by neurofilaments and desmin on terminal differentiation. Nestin is also expressed in odontoblasts from early to late developmental stages but it is unknown what kind of protein interacts with nestin. If podoplanin constructs cytoskeleton with nestin in the tooth germ, the expression and reduction of nestin may correspond with those of podoplanin at the development stages.

This study was aimed to investigate the relation between odontogenesis and the distribution of podoplanin-expressing cells in developing tooth germ.

II. Materials and Methods

Subjects

ICR mice from pregnancy 7th to 14th and from 1st after birth to 15th (n=10) (Charles River Japan Inc., Yokohama, Japan) were used. Maxillary tissue including upper molar tooth germ was obtained after euthanasia by intraperitoneal injection with sodium pentobarbital (10 ml/kg, Nembutal, Abbott Laboratories, North Chicago, IL, USA). The protocol for animal use was reviewed and approved by the animal experiment committee of Fukuoka Dental College, Fukuoka, Japan. Coronal undecalcified frozen tissue sections (8 μm) of molar tooth region were cut in a cryostat and fixed in 100% methanol for 1 min at –20°C.

Immunostaining

Sections were treated with 1% goat serum (GS) diluted with 10 mM phosphate-buffered saline (PBS, pH 7.4) for 10 min at room temperature (RT) and were exposed by a cocktail of primary antibodies: 1 μg/ml of hamster anti-mouse podoplanin IgG (AngioBio Co., Del Mar, CA) and rabbit anti-mouse nestin antibody (Abcam Inc., Cambridge, MA) for 8 hr at 4°C. After treatment with first antibodies,

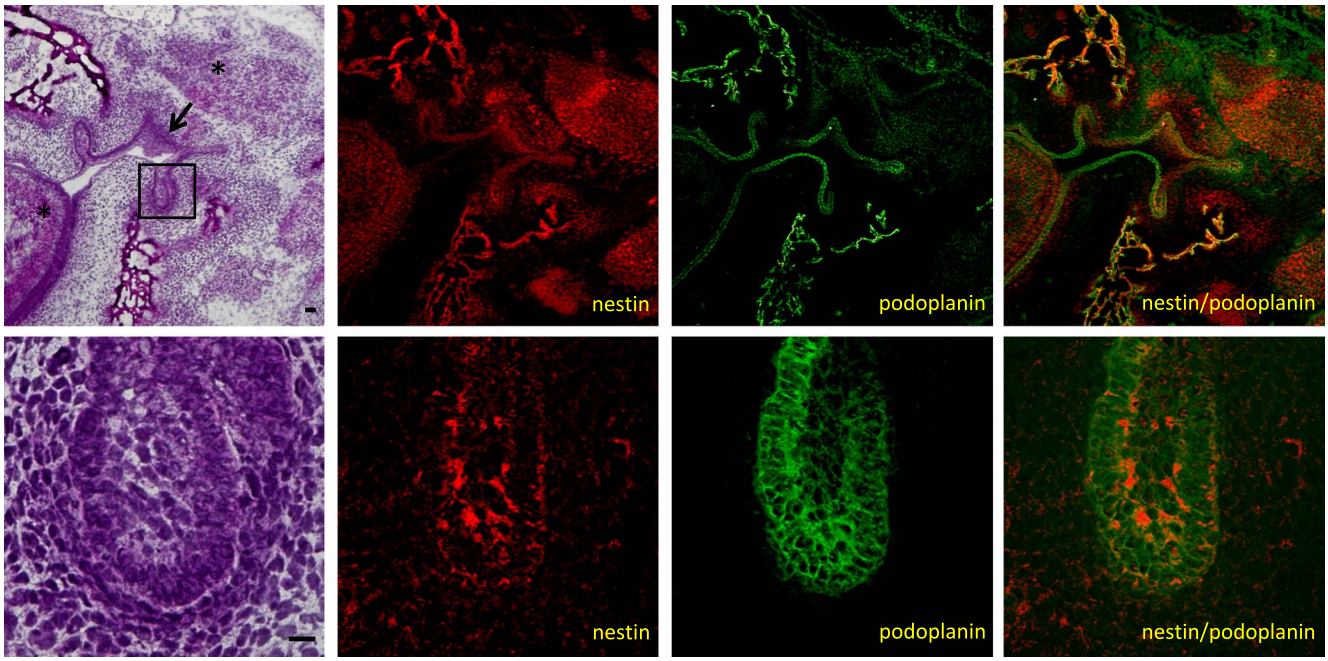

Fig. 1. Podoplanin expression in a mouse oral mucous epithelia and tooth germ at the bud stage (E14). Figures show coronal sections of molar tooth germ. Lower figures are the region highlighted by a box in the upper figure of H-E staining. Aggregated immature skeletal muscle cells in tongue and palate (asterisks), and oral mucous epithelia (arrow) are strongly stained in red with antibody for neural crest stem cell marker nestin. Cells that reacted with anti-nestin are scattered in the tooth bud and in mesenchymal cells are densely gathered around the tooth bud. Reaction products with anti-podoplanin (green) are detected strongly on the cell surface of oral mucous epithelia, and of the whole epithelia of the bud at cell-cell contacts, and are detected weakly on stellate reticulum in the center of tooth germ. The nestin-positive mesenchymal cells surrounding the tooth bud do not react with anti-podoplanin. Bar=100 μm.

sections were reacted with a cocktail of second antibodies: 1 μg/ml of AlexaFluor 488-conjugated goat anti-hamster IgG and AlexaFluor 568-conjugated goat anti-rabbit IgG (Molecular Probes, Invitrogen, Eugene, OR) in GS-PBS for 1 hr at RT, and then examined by fluorescence microscopy BZ-8100 (Keyence Corp., Osaka, Japan). The specificities of antibodies are described in our previous studies [8, 9, 21, 29].

III. Results

In a mouse tooth germ at the bud stage, the expression of nestin was observed in not only mesenchymal cells surrounding tooth buds but also in epithelial cells scattered in tooth buds, and in oral mucous epithelia (Fig. 1). Reaction products with anti-podoplanin were observed on oral mucous epithelial cells, salivary glands and bone [8, 9]. Strong expression of podoplanin was observed in tooth germ epithelial cells whereas podoplanin expression was not detected in nestin-positive mesenchymal cells surrounding the tooth bud (Fig. 1).

In mouse tooth germ at the cap stage, strong expression of nestin was observed in aggregated mesenchymal cells in the dental papilla (Fig. 2). It was also observed that reaction products with anti-nestin were present in the enamel organ at the inner enamel epithelial cells which lie above the aggregated dental papilla cells. Reaction products with anti-podoplanin were strongly detected on the inner and outer enamel epithelia at cell-cell contacts, and weakly on the loosely aggregated stellate reticulum in the center of the tooth germ but not in nestin-positive mesenchymal cells surrounding the tooth germ (Fig. 2).

In mouse tooth germ at the early bell stage, strong expression of nestin was observed in the enamel cord, aggregated stellate reticulum at the region adjacent to inner enamel epithelia, and odontoblasts in the tooth germ (Fig. 3). Strong expression of nestin was also found in the cervical loop where the outer enamel epithelium and the inner enamel epithelium cross over at the apical region of the tooth germ. Reaction products with anti-podoplanin were strongly detected in the enamel cord, and in the inner and outer enamel epithelia at the cervical loop. Immunoreaction with anti-podoplanin was lower in ameloblasts at the region which forms the top of the central cusp than in enamel epithelia at the cervical loop. In dental papilla only odontoblasts exhibit a strong immunoreaction to anti-podoplanin (Fig. 3).

In mouse tooth germ at the root formation stage with enamel and root formation, only weak reaction products with anti-nestin were found in odontoblasts forming the radicular dentin and in mesenchymal cells near the Hertwig epithelial sheath except for the cross reaction to bone and dental hard tissue (Fig. 4). Reaction products with anti-podoplanin were only found in odontoblasts forming radicular dentin and in Hertwig epithelial sheath except for the cross reaction of anti-podoplanin as well as that of anti-nestin (Fig. 4).

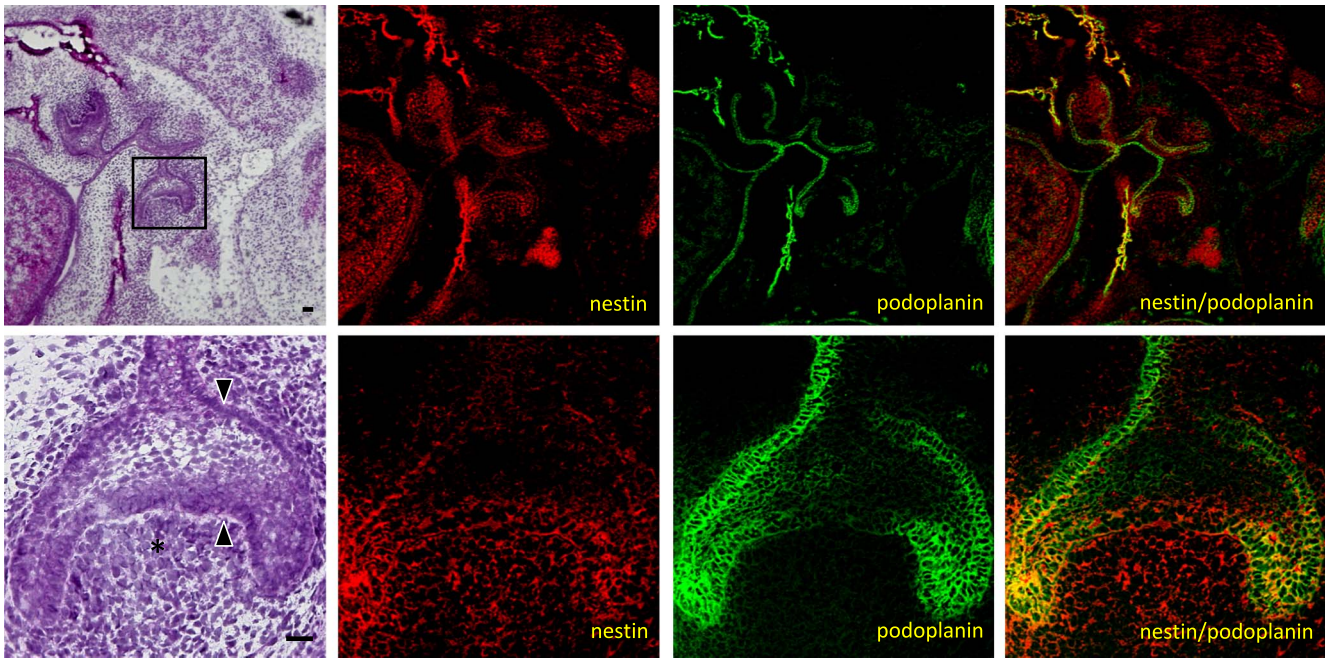

Fig. 2. Podoplanin expression in the mouse tooth germ at the cap stage (E16). Figures show coronal sections of molar tooth germ. Lower figures are the region highlighted by a box in the upper figure of H-E staining. It is found that aggregated mesenchymal cells of the dental papilla (asterisk) and inner enamel epithelia above aggregated dental papilla cells are strongly stained in red with antibody for nestin. Reaction products with anti-podoplanin (green) are found strongly on inner and outer enamel epithelia at cell-cell contacts (arrow), and weakly on the loosely aggregated stellate reticulum in the center of a tooth germ. The nestin-positive mesenchymal cells surrounding tooth germ do not react with anti-podoplanin. Bar=100 μm.

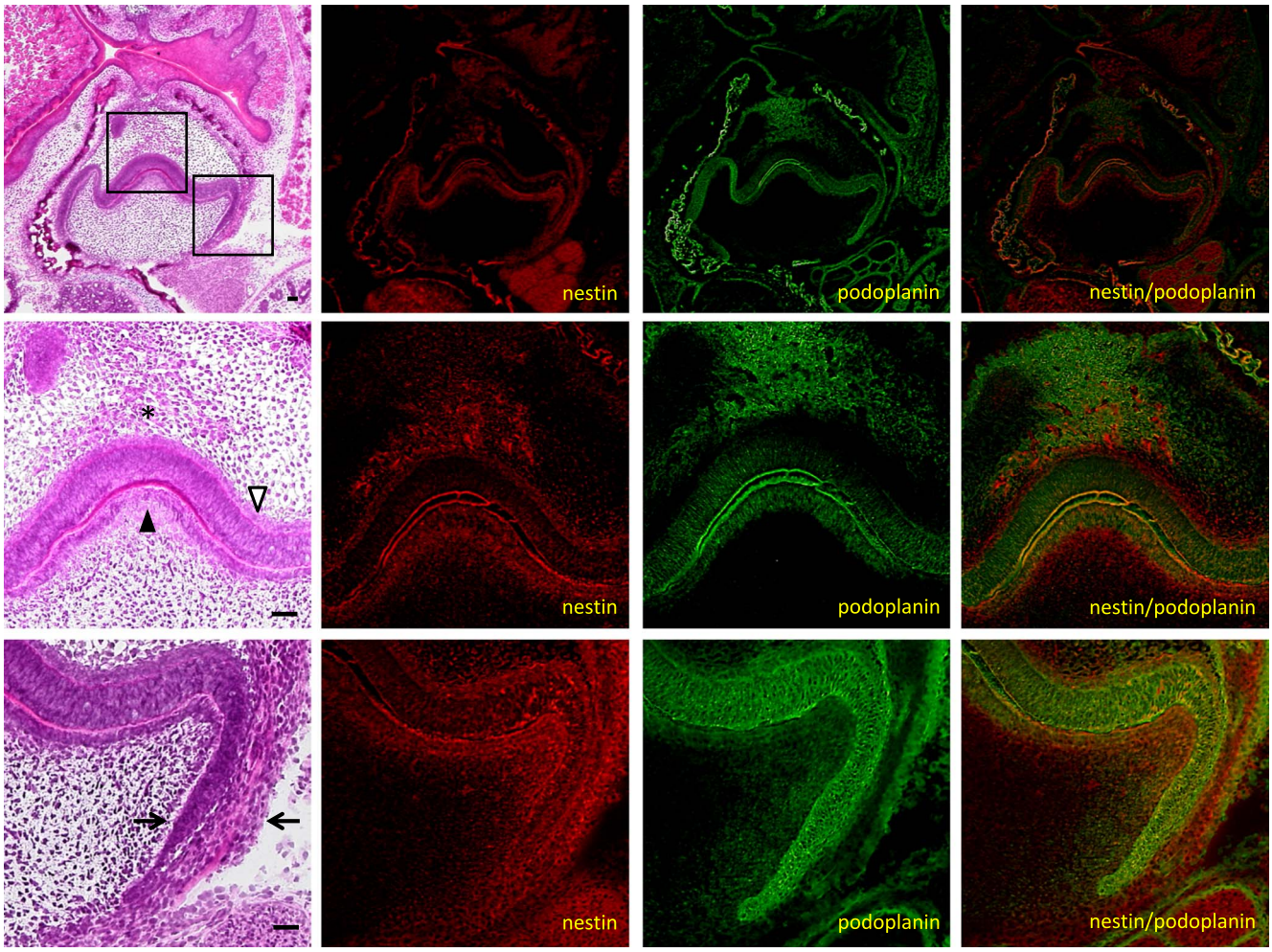

Fig. 3. Podoplanin expression in the mouse tooth germ at the early bell stage (E18). Figures show coronal sections of molar tooth germ. Middle and lower figures are regions highlighted by boxes in the upper figure of H-E staining. It is found that enamel cord (asterisk), aggregated stellate reticulum adjacent to inner enamel epithelia (outlined arrowhead), odontoblasts (arrowhead), and dental papilla cells underneath odontoblasts are strongly stained in red with antibody for nestin. Strong reaction products with anti-nestin are also found in the cervical loop where the outer enamel epithelium and the inner enamel epithelium come across at the apical region of the tooth germ (arrows). The enamel cord, and inner and outer enamel epithelia at the cervical loop are strongly stained with anti-podoplanin (green). Immunoreaction with anti-podoplanin is lower in ameloblasts at the top of central cusp than in enamel epithelia at the cervical loop. In the dental papilla only odontoblasts are stained by anti-podoplanin. Bar=100 μm.

IV. Discussion

According to a recent report the expression of nestin is found in aggregated immature skeletal muscle cells in tongue and palate [12], while the present study shows that nestin is expressed in both the mesenchymal and epithelial cells of the mouse tooth germ at the bud stage (Fig. 1). It is thought that epithelial cells of the tooth germ in the early developmental stage acquire the ability to express the neural crest stem cell marker nestin. The present study showed that podoplanin was expressed in oral mucosa and tooth bud epithelial cells but not in nestin-positive mesenchymal cells surrounding the tooth bud. It is thought that the ability of oral mucous epithelial cells to express podoplanin is maintained in tooth bud epithelial cells. In mouse tooth germ at the cap stage, both dental papilla mesenchymal cells and inner enamel epithelial cells expressed nestin, while inner and outer enamel epithelia expressed podoplanin, suggesting that inner enamel epithelia have the ability to express both nestin and podoplanin at the cap stage (Fig. 2). In mouse tooth germ at the early bell stage, both nestin and podoplanin were strongly expressed in the enamel cord, cervical loop enamel epithelia, and odontoblasts whereas the immunoreaction with both anti-nestin and anti-podoplanin was lower in ameloblasts than in enamel epithelia (Fig. 3). At the root formation stage nestin and podoplanin were weakly expressed only in odontoblasts forming radicular dentin while the Hertwig epithelial sheath expressed podoplanin but not nestin (Fig. 4). These results suggest that proliferating enamel epithelia and odontoblasts transiently exhibit the ability to produce nestin, and that the ability is reduced with differentiation (Table 1). The expression ability of

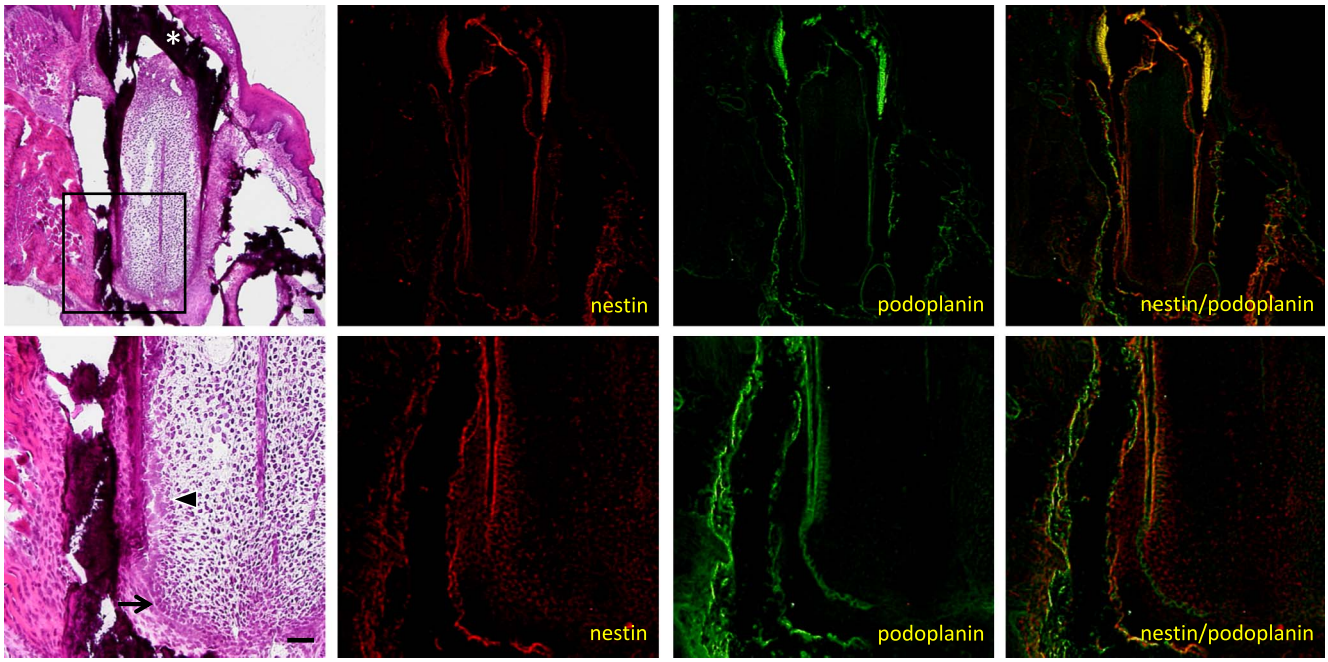

Fig. 4. Podoplanin expression in the mouse tooth germ at the root formation stage with enamel and root formation (P12). Figures show coronal undecalcified frozen sections of molar tooth germ. Lower figures are regions highlighted by a box in the upper figure of H-E staining. Reaction products with anti-nestin are only found in odontoblasts with radicular dentin formation (arrowhead) and in mesenchymal cells near Hertwig epithelial sheath (arrow). Reaction products with anti-podoplanin are only found in odontoblasts with generating radicular dentin and in the Hertwig epithelial sheath. There is a cross reaction with both anti-nestin and anti-podoplanin at the edge of the alveolar bone, dentin, and enamel. Bar=100 μm.

Table 1. *Expression of nestin and podoplanin in the mouse tooth germ cells*

	Epithelia			Mesenchyma		
Bud stage	Oral mucous epithelia	N	+	Mesenchymal cells surrounding tooth bud	N	+
		P	+			
	Tooth bud epithelia	N	Scattered		P	–
		P	+			
Cap stage	Inner and outer enamel epithelia	N	Scattered	Dental papilla	N	+
		P	+			
	Stellate reticulum	N	Weakly		P	–
		P	Center			
Early bell stage	Inner and outer enamel epithelia	N	+	Odontoblasts	N	+
		P	+			
	Enamel cord	N	+		P	+
		P	+			
Late bell stage	Hertwig epithelial sheath	N	–	Coronal odontoblasts	N	–
					P	–
		P	+	Odontoblasts forming radicular dentin	N	+
					P	+

The expression of nestin (N) in tooth germ epithelia was found partially in tooth bud epithelia and was detected strongly at the early bell stage, but was not found at the root formation stage. The expression of nestin in tooth germ mesenchymal cells was weakly found around tooth buds and in dental palilla, and was detected strongly in odontoblasts, especially at the early bell stage, but vanished with dentin completion. The expression of podoplanin (P) in tooth germ epithelia was found in tooth bud epithelia continuously from oral epithelia and was detected strongly at the early bell stage, but was only found in the Hertwig sheath at the late bell stage. The expression of podoplanin in tooth germ mesenchymal cells was only found in odontoblasts, and was detected especially strongly at the early bell stage, but vanished with dentin completion.

podoplanin in enamel epithelia appears to be maintained as a characteristic of the oral epithelium (Table 1).

Furthermore, only odontoblasts expressed podoplanin in dental papilla mesenchymal cells and the expression was reduced with the completion of a tooth (Figs. 3, 4) (Table 1). Podoplanin induces an actin cytoskeleton rearrangement dependent on the binding activity with a cytoplasmic linker protein ezrin. Podoplanin up-regulates the Ras homolog gene family, member A (RhoA)-GTPase activity resulting in the ezrin phosphorylation by Rho-associated coiled coil-containing protein kinase 1 [17, 33, 39]. Phosphorylated ezrin intermediates the connection between membrane proteins and F-actin, and the increase of podoplanin at the cell membrane promotes the formation of membrane-actin structures critical for cell shape and induces plasma membrane extension based on the cytoskeleton rearrangement [2, 5, 6, 15, 27–29]. Although it is unclear what molecules conjugate with podoplanin in odontoblasts because of the absence of usual expression of ezrin in mesenchymal cells, it is thought that the induction of podoplanin in odontoblasts contributes to form the cytoskeleton by binding with some intermediate filaments.

In this study, the expression of nestin was clearly found in the tooth germ. Nestin is an intermediate filament expressed in proliferating cells during early stages of development of the central nervous system, peripheral nervous system and myogenic tissue, and is also known as a useful marker of migrating cells. Nestin is induced dependent on the degree of cell differentiation and is replaced by tissue-specific intermediate filament proteins [20]. In this study, neither expression and reduction of nestin completely corresponded with those of podoplanin on tooth germ epithelial cells, but had good agreement with those on odontoblasts (Table 1). Since nestin was exclusively expressed in odontoblasts and epithelial cells of developing tooth germ before completion of dentin, it is thought that nestin may transiently function to form a cytoskeleton in tooth germ cells. The induction of podoplanin in odontoblasts may be involved in the arrangement of the nestin cytoskeleton and the podoplanin-nestin complex may play a role in the extension of the odontoblastic fibers.

V. References

1. About, I., Laurent-Maquin, D., Lendahl, U. and Mitsiadis, T. A. (2000) Nestin expression in embryonic and adult human teeth under normal and pathological conditions. *Am. J. Pathol.* 157; 287–295.
2. Belkina, N. V., Liu, Y., Hao, J. J., Karasuyama, H. and Shaw, S. (2009) LOK is a major ERM kinase in resting lymphocytes and regulates cytoskeletal rearrangement through ERM phosphorylation. *Proc. Natl. Acad. Sci. U S A* 106; 4707–4712.
3. Breiteneder-Geleff, S., Matsui, K., Soleiman, A., Meraner, P., Poczewski, H., Kalt, R., Schaffner, G. and Kerjaschki, D. (1997) Podoplanin, novel 43-kd membrane protein of glomerular epithelial cells, is down-regulated in puromycin nephrosis. *Am. J. Pathol.* 151; 1141–1152.
4. Dobbs, L. G., Williams, M. C. and Gonzalez, R. (1988) Monoclonal antibodies specific to apical surfaces of rat alveolar type I cells bind to surfaces of cultured, but not freshly isolated, type II cells. *Biochim. Biophys. Acta* 970; 146–156.
5. Endo, K., Kondo, S., Shackleford, J., Horikawa, T., Kitagawa, N., Yoshizaki, T., Furukawa, M., Zen, Y. and Pagano, J. S. (2009) Phosphorylated ezrin is associated with EBV latent membrane protein 1 in nasopharyngeal carcinoma and induces cell migration. *Oncogene* 28; 1725–1735.
6. Gautreau, A., Poullet, P., Louvard, D. and Arpin, M. (1999) Ezrin, a plasma membrane-microfilament linker, signals cell survival through the phosphatidylinositol 3-kinase/Akt pathway. *Proc. Natl. Acad. Sci. U S A* 96; 7300–7305.
7. Gröger, M., Loewe, R., Holnthoner, W., Embacher, R., Pillinger, M., Herron, S. G., Wolff, K. and Petzelbauer, P. (2004) Il-3 induces expression of lymphatic markers Prox-1 and podoplanin in human endothelial cells. *J. Immunol.* 173; 7161–7169.
8. Hata, M., Ueki, T., Sato, A., Kojima, H. and Sawa, Y. (2008) Expression of podoplanin in the mouse salivary glands. *Arch. Oral Biol.* 53; 835–841.
9. Hata, M., Amano, I., Tsuruga, E., Kojima, H. and Sawa, Y. (2010) Immunoelectron microscopic study of podoplanin localization in mouse salivary gland myoepithelium. *Acta Histochem. Cytochem.* 43; 77–82.
10. Hong, Y. K., Harvey, N., Noh, Y. H., Schacht, V., Hirakawa, S. and Detmar, M. (2002) Prox1 is a master control gene in the program specifying lymphatic endothelial cell fate. *Dev. Dyn.* 225; 351–357.
11. Kato, Y., Kaneko, M. K., Kunita, A., Ito, H., Kameyama, A., Ogasawara, S., Matsuura, N., Hasegawa, Y., Suzuki-Inoue, K., Inoue, O., Ozaki, Y. and Narimatsu, H. (2008) Molecular analysis of the pathophysiological binding of the platelet aggregation-inducing factor podoplanin to the C-type lectin-like receptor CLEC-2. *Cancer Sci.* 99; 54–61.
12. Kobayashi, M., Sjoberg, G., Soderhall, S., Lendahl, U., Sandstedt, B. and Sejersen, T. (1998) Pediatric rhabdomyosarcomas express the intermediate filament nestin. *Pediat. Res.* 43; 386–392.
13. Koop, K., Eikmans, M., Wehland, M., Baelde, H., Ijpelaar, D., Kreutz, R., Kawachi, H., Kerjaschki, D., de Heer, E. and Bruijn, J. A. (2008) Selective loss of podoplanin protein expression accompanies proteinuria and precedes alterations in podocyte morphology in a spontaneous proteinuric rat model. *Am. J. Pathol.* 173; 315–326.
14. Kriehuber, E., Breiteneder-Geleff, S., Groeger, M., Soleiman, A., Schoppmann, S. F., Stingl, G., Kerjaschki, D. and Maurer, D. (2001) Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages. *J. Exp. Med.* 194; 797–808.
15. Lan, M., Kojima, T., Murata, M., Osanai, M., Takano, K., Chiba, H. and Sawada, N. (2006) Phosphorylation of ezrin enhances microvillus length via a p38 MAP-kinase pathway in an immortalized mouse hepatic cell line. *Exp. Cell Res.* 312; 111–120.
16. Marsee, D. K., Pinkus, G. S. and Hornick, J. L. (2009) Podoplanin (D2-40) is a highly effective marker of follicular dendritic cells. *Appl. Immunohistochem. Mol. Morphol.* 17; 102–107.
17. Martín-Villar, E., Megías, D., Castel, S., Yurrita, M. M., Vilaró, S. and Quintanilla, M. (2006) Podoplanin binds ERM proteins to activate RhoA and promote epithelial-mesenchymal transition. *J. Cell Sci.* 119; 4541–4553.
18. Matsui, K., Breiteneder-Geleff, S. and Kerjaschki, D. (1998) Epitope specific antibodies to the 43 kDa glomerular membrane protein podoplanin cause proteinuria and rapid flattening of podocytes. *J. Am. Soc. Nephrol.* 9; 2013–2026.
19. Matsui, K., Breitender-Geleff, S., Soleiman, A., Kowalski, H. and Kerjaschki, D. (1999) Podoplanin, a novel 43-kDa membrane protein, controls the shape of podocytes. *Nephrol. Dial. Transplant* 14 Suppl 1; 9–11.
20. Michalczyk, K. and Ziman, M. (2005) Nestin structure and predicted function in cellular cytoskeletal organisation. *Histol.*

Histopathol. 20; 665–671.
21. Noda, Y., Amano, I., Hata, M., Kojima, H. and Sawa, Y. (2010) Immunohistochemical examination on the distribution of cells expressed lymphatic endothelial marker podoplanin and LYVE-1 in the mouse tongue tissue. *Acta Histochem. Cytochem.* 43; 61–68.
22. Ogawa, R., Saito, C., Jung, H. S. and Ohshima, H. (2006) Capacity of dental pulp differentiation after tooth transplantation. *Cell Tissue Res.* 326; 715–724.
23. Ordóñez, N. G. (2005) D2-40 and podoplanin are highly specific and sensitive immuno-histochemical markers of epithelioid malignant mesothelioma. *Hum. Pathol.* 36; 372–380.
24. Ordóñez, N. G. (2006) Podoplanin: a novel diagnostic immunohistochemical marker. *Adv. Anat. Pathol.* 13; 83–88.
25. Petrova, T. V., Makinen, T., Makela, T. P., Saarela, J., Virtanen, I., Ferrell, R. E., Finegold, D. N., Kerjaschki, D., Ylä-Herttuala, S. and Alitalo, K. (2002) Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. *EMBO J.* 21; 4593–4599.
26. Raica, M., Cimpean, A. M. and Ribatti, D. (2008) The role of podoplanin in tumor progression and metastasis. *Anticancer Res.* 28; 2997–3006.
27. Ramirez, M. I., Millien, G., Hinds, A., Cao, Y. X., Seldin, D. C. and Williams, M. C. (2003) T1alpha, a lung type I cell differentiation gene, is required for normal lung cell proliferation and alveolous formation at birth. *Dev. Biol.* 256; 61–72.
28. Rishi, A. K., Joyce-Brady, M., Fisher, J., Dobbs, L. G., Floros, J., VanderSpek, J., Brody, J. S. and Williams, M. C. (1995) Cloning, characterization, and development expression of a rat lung alveolar type I cell gene in embryonic endodermal and neural derivatives. *Dev. Biol.* 167; 294–306.
29. Sawa, Y., Iwasawa, K. and Ishikawa, H. (2008) Expression of podoplanin in the mouse tooth germ and apical bud cells. *Acta Histochem. Cytochem.* 41; 121–126.
30. Sawa, Y. (2010) New trends in the study of podoplanin as a cell morphological regulator. *Japanese Dent. Sci. Rev.* 46; 165–172.
31. Schacht, V., Ramirez, M. I., Hong, Y. K., Hirakawa, S., Feng, D., Harvey, N., Williams, M., Dvorak, A. M., Dvorak, H. F., Oliver, G. and Detmar, M. (2003) T1α/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. *EMBO J.* 22; 3546–3556.
32. Schacht, V., Dadras, S. S., Johnson, L. A., Jackson, D. G., Hong, Y. K. and Detmar, M. (2005) Up-regulation of the lymphatic marker podoplanin, a mucin-type transmembrane glycoprotein, in human squamous cell carcinomas and germ cell tumors. *Am. J. Pathol.* 166; 913–921.
33. Scholl, F. G., Gamallo, C., Vilar, S. and Quintanilla, M. (1999) Identification of PA2.26 antigen as a novel cell-surface mucin-type glycoprotein that induces plasma membrane extensions and increased motility in keratinocytes. *J. Cell Sci.* 112; 4601–4613.
34. Suzuki-Inoue, K., Kato, Y., Inoue, O., Kaneko, M. K., Mishima, K., Yatomi, Y., Yamazaki, Y., Narimatsu, H. and Ozaki, Y. (2007) Involvement of the snake toxin receptor CLEC-2, in podoplanin-mediated platelet activation, by cancer cells. *J. Biol. Chem.* 282; 25993–26001.
35. Terling, C., Rass, A., Mitsiadis, T. A., Fried, K., Lendahl, U. and Wroblewski, J. (1995) Expression of the intermediate filament nestin during rodent tooth development. *Int. J. Dev. Biol.* 39; 947–956.
36. Wetterwald, A., Hoffstetter, W., Cecchini, M. G., Lanske, B., Wagner, C., Fleisch, H. and Atkinson, M. (1996) Characterization and cloning of the E11 antigen, a marker expressed by rat osteoblasts and osteocytes. *Bone* 18; 125–132.
37. Williams, M. C., Cao, Y., Hinds, A., Rishi, A. K. and Wetterwald, A. (1996) T1a protein is developmentally regulated and expressed by alveolar type I cells, choroid plexus and ciliary epithelia of adult rats. *Am. J. Respir. Cell. Mol. Biol.* 14; 577–585.
38. Yu, H., Gibson, J. A., Pinkus, G. S. and Hornick, J. L. (2007) Podoplanin (D2-40) is a novel marker for follicular dendritic cell tumors. *Am. J. Clin. Pathol.* 128; 776–782.
39. Yu, Y., Khan, J., Khanna, C., Helman, L., Meltzer, P. S. and Merlino, G. (2004) Expression profiling identifies the cytoskeletal organizer ezrin and the developmental homeoprotein Six-1 as key metastatic regulators. *Nat. Med.* 10; 175–181.
40. Zayed, A. E., Abd-Elnaeim, M. M., Abd-Elghaffar, S. K., Hild, A., Brehm, R. and Steger, K. (2007) Prenatal development of murine gonads with special reference to germ cell differentiation: a morphological and immunohistochemical study. *Andrologia* 39; 93–100.